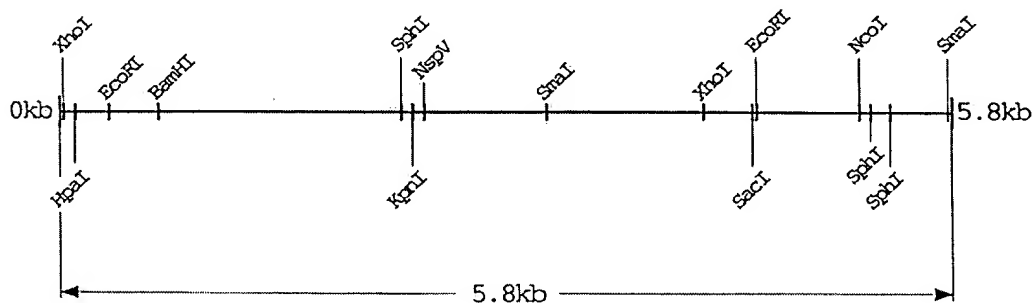


IN THE CLAIMS:

Please amend Claims 3, 6, 9, 10, 13, 17, 22, 28, 34 and 55 as shown below.

The claims, as pending in the subject application, read as follows:

6. 1. (Previously Presented) An isolated DNA fragment of about 5.8 Kb containing a toluene monooxygenase gene, having 1 BamHI restriction site, 2 EcoRI restriction sites, 1 HpaI restriction site, 1 KpnI restriction site, 1 NcoI restriction site, 1 NspV restriction site, 1 SacI restriction site, 2 SmaI restriction sites, 3 SphI restriction sites, 2 XhoI restriction sites, no ClaI restriction site, no DraI restriction site, no EcoRV restriction site, no HindIII restriction site, no NdeI restriction site, no NheI restriction site, no PvuII restriction site, no ScaI restriction site, no Sse8387I restriction site, no StuI restriction site, and no XbaI restriction site, and having a restriction map of:



, said isolated DNA-fragment derived from Burkholderia cepacia KK01.

2. (Previously Presented) A DNA fragment isolated from Burkholderia cepacia KKOI wherein the DNA fragment has a nucleotide sequence of SEQ ID NO: 1.

3. (Currently Amended) An isolated DNA fragment cloned from SEQ ID NO. 1 having a nucleotide sequence ~~that hybridizes under stringent conditions to a hybridization probe with a nucleotide sequence consisting~~ of SEQ ID NO. 1, or a complement of SEQ ID NO. 1, wherein said nucleotide sequence includes deletion, substitution or addition of one or more bases from cloning, said DNA fragment encoding a protein having a toluene monooxygenase activity.

4. (Previously Presented) A recombinant DNA comprising a vector enabling maintenance or replication in a host, said vector including a DNA fragment according to any one of claims 1 to 3.

5. (Previously Presented) The recombinant DNA according to Claim 4, wherein the vector can be maintained or replicated in a bacterium.

6. (Currently Amended) An isolated DNA fragment containing a region encoding a toluene monooxygenase, the region comprising a first sequence encoding a polypeptide TomL having an amino acid sequence of SEQ ID NO: 3, a second sequence encoding a polypeptide TomM having an amino acid sequence of SEQ ID NO: 4, a third sequence encoding a polypeptide TomN having an amino acid sequence of SEQ ID NO: 5, a

fourth sequence encoding a polypeptide TomO having an amino acid sequence of SEQ ID NO: 6, and a fifth sequence encoding a polypeptide TomP having an amino acid sequence of SEQ ID NO: 7 ~~of the Sequence Listing~~, and the first to fifth sequences are aligned so that expressed TomL - TomP polypeptides can form said toluene monooxygenase protein.

7. (Previously Presented) An isolated DNA fragment according to claim 6, wherein no spacer sequence is present between the first to fifth sequences or at least one spacer sequence is present between the first to fifth sequences.

8. (Previously Presented) An isolated DNA fragment according to claim 6 or 7, further comprising a sequence encoding a polypeptide TomQ having an amino acid sequence of SEQ ID NO: 8.

9. (Currently Amended) An isolated DNA fragment cloned from SEQ ID NO: 1 containing a region encoding a toluene monooxygenase, wherein the region comprises a first sequence ~~that hybridizes under stringent conditions to a hybridization probe of which~~ having a nucleotide sequence consists of 463..1455 portion of SEQ ID NO: 1 or a complement thereof, encoding a polypeptide TomL having an amino acid sequence of SEQ ID NO:3, a second sequence ~~that hybridizes under stringent conditions to a hybridization probe of which~~ having a nucleotide sequence consists of 1495..1761 portion of SEQ ID NO: 1 or a complement thereof, encoding a polypeptide TomM having an amino acid sequence of SEQ ID NO: 4, a third sequence ~~that hybridizes under stringent conditions to a hybridization probe of which~~ having a nucleotide sequence consists of 1803..3350 portion of SEQ ID NO: 1 or a complement thereof, encoding a polypeptide TomN having an amino acid of SEQ ID NO: 5,

a fourth sequence ~~that hybridizes under stringent conditions to a hybridization probe of which~~ having a nucleotide sequence consists of 3428..3781 portion of SEQ ID NO: 1 or a complement thereof, encoding a polypeptide TomO having an amino acid sequence of SEQ ID NO: 6, and a fifth sequence ~~that hybridizes under stringent conditions to a hybridization probe of which~~ having a nucleotide sequence consists of 3810..4871 portion of SEQ ID NO: 1 or a complement thereof, encoding a polypeptide TomP having an amino acid sequence of SEQ ID NO: 7, ~~and wherein~~ the first to fifth sequences are aligned so that expressed polypeptides can form said toluene monooxygenase protein, and wherein the first to fifth sequences include deletion, substitution or addition of one or more bases from cloning.

10. (Currently amended) An isolated DNA fragment cloned from the 234...434 portion of SEQ ID NO: 1 comprising a region ~~that hybridizes under stringent conditions to a hybridization probe of which~~ having a nucleotide sequence consists of 234...443 portion of SEQ ID NO: 1 or a complement thereof, encoding a polypeptide having a property ~~of enhanced~~ to enhance toluene monooxygenase activity, wherein the isolated DNA fragment includes deletion, substitution or addition of one or more bases from cloning.

11. (Previously Presented) A recombinant DNA comprising a vector, wherein said vector contains a promoter which is functionally ligated to a DNA fragment according to any one of claims 6, 7 or 9 to enable expression of the toluene monooxygenase encoded by the DNA fragment.

12. (Original) The recombinant DNA according to claim 11 wherein the promoter and the vector can function in a bacterium.

13. (Currently Amended) A recombinant DNA comprising an expression vector comprising a first promoter and a first DNA fragment cloned from the 234...443 portion of SEQ ID NO: 1 comprising a region ~~that hybridizes under stringent conditions to a hybridization probe of which~~ having a nucleotide sequence ~~consists~~ of 234...443 portion of SEQ ID NO: 1 or a complement thereof, encoding a polypeptide having a property of ~~enhanced~~ to enhance toluene monooxygenase activity, a second promoter and the DNA fragment according to any one of Claims 6, 7, and 9, wherein the first DNA fragment is functionally linked to the first promoter, and the second DNA fragment is functionally linked to the second promoter, and wherein the first and second DNA fragments include deletion, substitution or addition of one or more bases from cloning.

14. (Original) The recombinant DNA according to claim 13, wherein the first and second promoters and the vector can function in a bacterium.

15. (Previously Presented) A transformant obtained by introducing a recombinant DNA into a host microorganism, the recombinant DNA comprising a vector enabling maintenance or replication in a host and a DNA fragment of about 5.8 Kb containing a toluene monooxygenase gene having 1 BamHI restriction site, 2 ECOR1 restriction sites, 1 HpaI restriction site, 1 KpnI restriction site, 1 NcoI restriction site, 1 NspV restriction site, 1 SacI restriction site, 2 SmaI restriction sites, 3 SphI restriction sites, 2 XhoI restriction sites, no ClaI restriction site, no DraI restriction site, no EcoRV restriction site, no HindIII restriction site, no NdeI restriction site, no NheI restriction site, no PvuII restriction site, no Scal

restriction site, no Sse83871 restriction site, no StuI restriction site, and no XbaI restriction site, said DNA-fragment derived from Burkholderia cepacia KKOl.

16. (Original) The transformant according to claim 15, wherein the host microorganism is a bacterium.

17. (Currently Amended) A transformant obtained by introducing a recombinant DNA into a host microorganism, where the recombinant DNA comprises a vector enabling maintenance or replication in a host, said vector including a DNA fragment cloned from SEQ ID NO: 1 ligated thereto having a ~~sequence that hybridizes under stringent conditions to a hybridization probe of which~~ nucleotide sequence consists of SEQ ID NO: 1 or a complement of SEQ ID NO: 1 and encoding an active toluene monooxygenase, wherein the DNA fragment is 4.9 kb or less encoding [[a]] an active toluene monooxygenase, and wherein the DNA fragment includes deletion, substitution or addition of one or more bases from cloning.

18. (Original) The transformant according to claim 17, wherein the host microorganism is a bacterium.

19. (Previously Presented) A transformant obtained by introducing a recombinant DNA comprising a vector, a promoter and a DNA fragment into a host microorganism where the DNA fragment contains a region encoding a toluene monooxygenase, the region comprising a first sequence encoding a polypeptide TomL having an amino acid sequence of SEQ ID NO: 3, a second sequence encoding a polypeptide TomM

having an amino acid sequence of SEQ ID NO: 4, a third sequence encoding a polypeptide TomN having an amino acid sequence of SEQ ID NO: 5, a fourth sequence encoding a polypeptide TomO having an amino acid sequence of SEQ ID NO: 6, and a fifth sequence encoding a polypeptide TomP having an amino acid sequence of SEQ ID NO: 7, and the first to fifth sequences are aligned so that expressed TomL - TomP polypeptides can form said toluene monooxygenase protein;

wherein the promoter and the DNA fragment are functionally linked enabling expression of the toluene monooxygenase protein encoded by the DNA fragment.

20. (Original) The transformant according to claim 19, wherein said host microorganism is a bacterium.

21. (Previously Presented) A method for producing a toluene monooxygenase, comprising the steps of:

culturing a transformant according to any one of claims 15, 17 and 19 in a medium; and collecting the expressed toluene monooxygenase.

22. (Currently Amended) A method for degrading at least one of a chlorinated aliphatic hydrocarbon compound and an aromatic compound in a medium comprising a step of ~~degrading~~ contacting at least one of a chlorinated aliphatic hydrocarbon compound and an aromatic compound by using the transformant according to any one of claims 15, 17 and 19.


23. (Original) The degradation method according to claim 22, wherein the medium is an aqueous medium.

24. (Original) The degradation method according to claim 22, wherein the medium is soil.

25. (Original) The degradation method according to claim 22, wherein the medium is air.

26. (Original) The degradation method according to claim 22, wherein the chlorinated aliphatic hydrocarbon compound is either trichloroethylene (TCE) or dichloroethylene (DCE).

27. (Previously Presented) The degradation method according to claim 22, wherein the aromatic compound is selected from the group consisting of toluene, benzene, phenol, and cresol.

 28. (Currently Amended) A method for cleaning a medium polluted with at least one of a chlorinated aliphatic hydrocarbon compound and aromatic compound comprising a step of degrading contacting at least one of a chlorinated aliphatic hydrocarbon compound and an aromatic compound using the transformant according to any one of claims 15, 17 and 19.



29. (Original) The cleaning method according to claim 28 wherein the medium is an aqueous medium.

30. (Original) The cleaning method according to claim 28 wherein the medium is soil.

31. (Original) The cleaning method according to claim 28 wherein the medium is air.

32. (Original) The cleaning method according to claim 28 wherein the chlorinated aliphatic hydrocarbon compound is either trichloroethylene (TCE) or dichloroethylene (DCE).

33. (Previously Presented) The cleaning method according to claim 28 wherein, the aromatic compound is selected from the group consisting of toluene, benzene, phenol, and cresol.

34. (Currently Amended) A method for remedying an environment polluted with a pollutant being a chlorinated aliphatic hydrocarbon compound or an aromatic compound, comprising a step of degrading contacting the pollutant by using the transformant according to any one of claims 15, 17 and 19.

35. (Original) The remediation method according to claim 34 wherein the environment is made of an aqueous medium.

36. (Original) The remediation method according to claim 35 wherein the polluted aqueous medium is brought into contact with a carrier holding the transformant.

37. (Original) The remediation method according to claim 36 wherein the contact is carried out by placing the carrier holding the transformant in a container, introducing the polluted aqueous medium from one side of the container, and discharging the remedied aqueous medium from another side.

38. (Original) The remediation method according to claim 34, wherein the environment is made of soil.

39. (Original) The remediation method according to claim 38 being carried out by introducing an aqueous medium containing the transformant into the polluted soil and supplying nutrients and/or oxygen for proliferation of the transformant in the polluted soil.

40. (Original) The remediation method according to claim 39 wherein the transformant is introduced in the soil with applying pressure through an injection well provided in the polluted soil.

41. (Original) The remediation method according to claim 38 wherein the polluted soil is introduced in a liquid phase containing the transformant.

42. (Original) The remediation method according to claim 38 wherein the polluted soil is brought into contact with a carrier holding the transformant.

43. (Original) The remediation method according to claim 34 wherein the environment is made of air.

44. (Original) The remediation method according to claim 43 wherein the polluted air is introduced into a liquid phase containing the transformant.

45. (Original) The remediation method according to claim 43 wherein the polluted air is brought into contact with a carrier holding the transformant.

46. (Original) The remediation method according to claim 45 wherein contact is carried out by placing the carrier holding the transformant in a container, introducing polluted air from one side of the container, and discharging cleaned air from another side.

47. (Original) The remediation method according to claim 34 wherein the chlorinated aliphatic hydrocarbon compound is either trichloroethylene (TCE) or dichloroethylene (DCE).

48. (Previously Presented) The remediation method according to claim 34, wherein the aromatic compound is selected from the group consisting of toluene, benzene, phenol, and cresol.

49 to 54 (Cancelled).

55. (Currently Amended) A recombinant DNA comprising an expression vector comprising a promoter, a first DNA fragment and a second DNA fragment, said first DNA fragment being a DNA fragment of any one of claims 6, 7 or 9, said second DNA fragment being a DNA fragment ~~comprising a region that hybridizes under stringent conditions to a hybridization probe of which~~ cloned from the 234...443 portion of SEQ ID NO: 1 having a nucleotide sequence ~~consists~~ of 234...443 portion of SEQ ID NO: 1 or a complement thereof, encoding a polypeptide having a property ~~of enhanced~~ to enhance toluene monooxygenase activity,

wherein the first DNA fragment containing ~~[[a]]~~ an active toluene monooxygenase encoding region of 4.9 kb or less is functionally connected to the promoter to express an active toluene monooxygenase, and the second DNA fragment is functionally connected to the promoter to express a property to enhance the toluene monooxygenase activity, and

wherein the first and second DNA fragments include deletion, substitution or addition of one or more bases from cloning.

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